PERMEABILITY AND FATIGUE IN MUSCLE AND ITS BEARING ON THE PROBLEM OF ION ANTAGONISM

E. GELLHORN [1]

(From the Department of Animal Biology, University of Oregon, Eugene, Oregon)

It has been shown by numerous investigators that stimulation of different tissues leads to an increase in permeability which is independent of the particular structure and function.[2] In the case of muscle, Weiss showed that even subminimal stimuli lead to an increase in permeability which was measured by an increase in the output of phosphoric acid by the muscle. When effective stimuli were applied, a close relationship between the intensity of fatigue and the increase in permeability was observed (Embden and Adler, 1922). Furthermore, there was a parallelism between the reversibility of fatigue and that of the changes in permeability. When the muscle recovered from fatigue and the original contractility was again obtained, the permeability was also normal, *i.e.*, no phosphoric acid was given off to the medium in which the muscle was suspended. But if no recovery took place, the output of phosphoric acid remained the same or even increased. These experiments prove that the physico-chemical changes in permeability play an important part in the fatigue problem. It seemed probable that fatigue might be delayed if it were possible to prevent or to diminish the increase in permeability which ordinarily accompanies fatigue. The experiments described in this paper show the correctness of the supposition. Fatigue can be delayed in a medium by which the permeability of the muscle is decreased. The efficiency of different ions in that respect and the general importance of these facts for the problem of ion antagonism will be discussed in this paper.

Method

Several hundred experiments on the sartorius muscle of *Rana esculenta* were performed from March 1929 to October 1930. The muscles, prepared in the usual manner, were immersed in an isotonic salt solution and suspended between two platinum electrodes connected with an isotonic lever which magnified the contractions seven times.

[1] Awarded the A. Cressy Morrison Prize in Experimental Biology for 1930 by the New York Academy of Sciences.

Aided by a grant from the Research Fund of the University of Oregon.

[2] Compare Gellhorn, 1929, pp. 166–197.

In the first group of experiments the platinum electrodes were carefully covered with lacquer (except on a point-like spot where the platinum was in contact with the muscle). The muscles were stimulated with rhythmical condenser discharges (apparatus of Scheminsky) 40–60 times per minute so that a decrease in the height of contraction occurred. The two sartorii of the same frog under the same conditions showed an identical course of fatigue (Gellhorn, 1930). This made it possible to study the influence of different ions. The solutions were made up of NaCl, KCl and $CaCl_2$ solutions of $\triangle = 0.39°$. In order to hold the conductivity of the liquid constant and avoid any possible effect on the strength of the stimulus, a part of the sodium chloride solution was replaced by an equal volume of isotonic $CaCl_2$ solution, and thus the effect of different $CaCl_2$ concentrations was studied.

In a second group of experiments another method was used. The sartorii were not stimulated in a liquid but in a moist chamber. The first series of stimuli was applied for five minutes and the muscle was then allowed to recover in an aërated salt solution. Afterward a second series of stimuli was applied until the muscle ceased to respond. The first series of stimuli was designed to show whether both sartorii had the same irritability and fatigability. Pairs of muscles which behaved alike in the first series of stimulation were the only ones considered as material for the determination of the influence of different salt solutions. The effect of these solutions was apparent in the course of fatigue during the second series of contractions. The advantage of this second method consists in the independence of the strength of the stimulus from the conductivity of the solution and furthermore in the exact proof of the identical behavior of the muscles before they were immersed in the salt solutions. In these experiments condenser discharges obtained from the apparatus of Scheminsky (1930) in its improved form were used. Maximal stimuli were given 90 times per minute. Between the first and the second series of stimulations the muscle remained unstimulated for nine minutes in the salt solutions. The experiments of the second group were performed with curarized and uncurarized muscles. The effect of Ca, Sr, Ba, and Mg was studied in reference to the fatigue of muscle.[3]

Experiments Performed with the First Method

Figure 1 shows the course of the height of contractions of the sartorii stimulated 40 times per minute for twelve minutes. One muscle was in NaCl + KCl + $CaCl_2$ solution in which these salts had

[3] After preparation the muscles remained in aërated Ringer's solution for 45–60 minutes before the experiment was begun.

the same concentration as in Ringer's solution, while the other muscle was bathed in $NaCl + KCl + CaCl_2$ which contained eight times as much $CaCl_2$ as the first one. It is apparent that, in spite of the identical height of the contractions at the beginning of the experiment, both

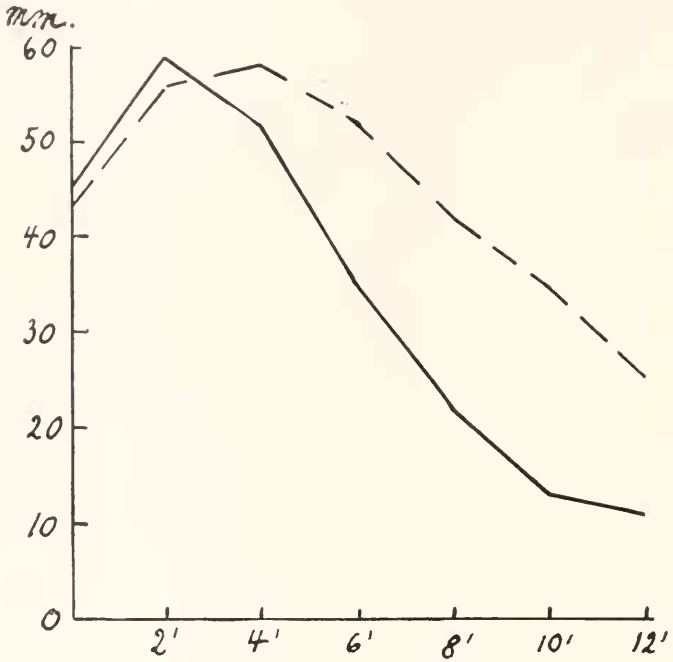

FIG. 1. The course of fatigue in a pair of sartorii stimulated in 0.111 M $NaCl + 10.2\ M \times 10^{-4}\ KCl + 8.1\ M \times 10^{-4}\ CaCl_2$ (——) and in 0.103 M $NaCl + 10.2\ M \times 10^{-4}\ KCl + 64.4\ M \times 10^{-4}\ CaCl_2$ (— — —).

Ordinate: height of contraction; Abscissa: time in minutes.

Maximal condenser discharges; frequency 40 per minute.

show the "treppe" in the same amount:—the muscle immersed in a solution with a high $CaCl_2$ concentration showed a lesser fatigue than the control muscle. In other words *an excess of $CaCl_2$ delays fatigue.* This fact, which was the basis for further experimentation, is illustrated by Fig. 2, which reproduces a series of experiments performed with six pairs of sartorii. The control muscle was always stimulated in 0.111M $NaCl + 0.00102M\ KCl + 0.00081M\ CaCl_2$, the other muscle in 0.00486M or 0.00972M $CaCl_2$, the KCl concentrations being identical in both cases and the NaCl slightly less in order to keep constant the osmotic pressure and conductivity of the solution. The contractions were registered at intervals of two minutes for a period of twelve minutes and the height of contraction of the muscle stimulated in the

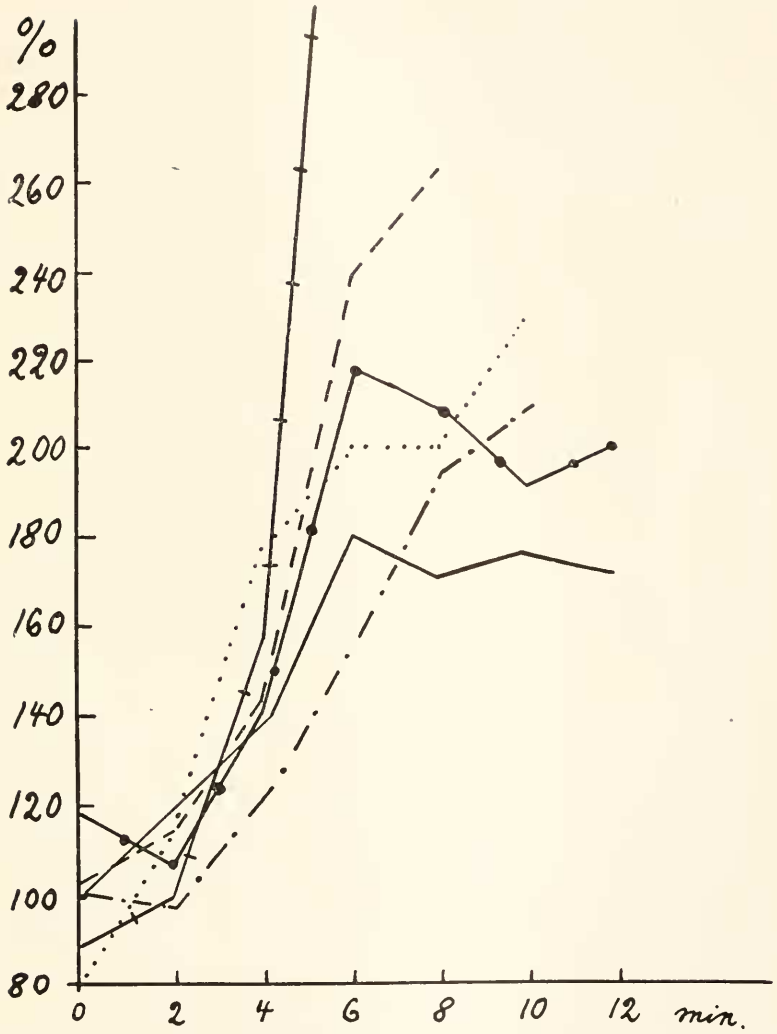

FIG. 2. The influence of different $CaCl_2$ concentrations on the course of fatigue in sartorius muscle. The height of contraction of the control muscle (stimulated in 0.111 M NaCl + 10.2 M $\times 10^{-4}$ KCl + 8.1 M $\times 10^{-4}$ $CaCl_2$) is taken as 100 and the percentage of the height of contraction of the muscle placed in a solution with higher $CaCl_2$ concentration is calculated.

——●—— } 0.106 M NaCl + 10.2 M $\times 10^{-4}$ KCl + 48.6 M $\times 10^{-4}$ $CaCl_2$.
— — — —

— · — · — } 0.0996 M NaCl + 10.2 M $\times 10^{-4}$ KCl + 97.2 M $\times 10^{-4}$ $CaCl_2$.
. . . . . .
—|—|—

higher $CaCl_2$ concentration was calculated as the percentage of the height of contraction of the control muscle. The figure shows that the height of contraction in the beginning of the experiment exhibited very slight variations if any, but in the further course of the experiment the deviations became progressively stronger and reached in most cases 100 per cent or more. This illustrates again the delay of fatigue in solutions with higher $CaCl_2$ concentrations and shows at the same time that the increase of $CaCl_2$ to twelve times its normal concentration (*i.e.*, the concentration in Ringer's solution) is still favorable for the preservation of contractility and the delay of fatigue.

The fundamental importance of this fact is apparent when the

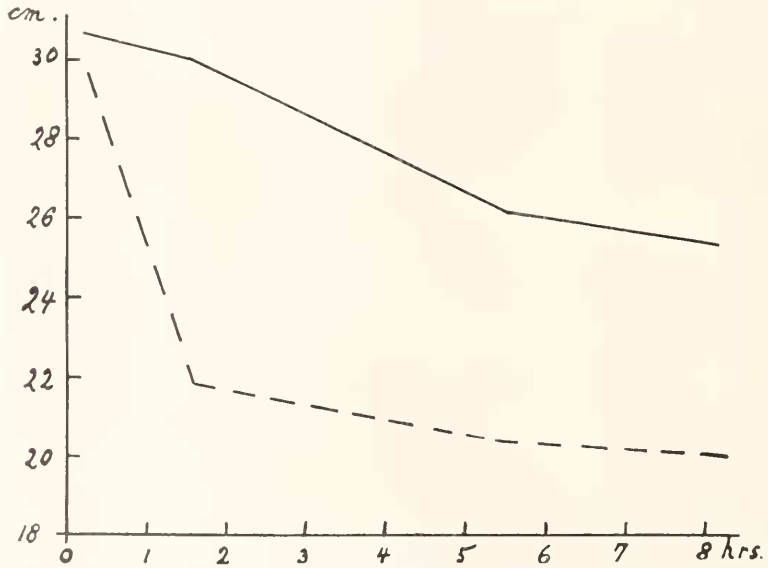

FIG. 3. The course of irritability (threshold) in unstimulated muscles. Ordinate: Distance between primary and secondary coil; abscissa: Time in hours.
——— 0.111 M NaCl + 10.2 M × 10^{-4} KCl + 8.1 M × 10^{-4} $CaCl_2$.
— — — 0.103 M NaCl + 10.2 M × 10^{-4} KCl + 64.4 M × 10^{-4} $CaCl_2$.

effect of the same solutions is studied on the irritability of the unstimulated muscle. As was to be expected, the solution with the "normal" $CaCl_2$ concentration is much more favorable to the preservation of irritability than a solution with higher $CaCl_2$ content (Fig. 3). Therefore the optimal conditions are different for the stimulated and unstimulated muscle. That is to say, *the laws of ion antagonism which lead to the discovery of the equilibrated solutions do not hold quantitatively for the stimulated muscle.* These experiments make probable an even

more general formulation that the efficiency of ions on cells depends upon their degree of permeability.

Experiments Performed with the Second Method

In order to check these results more than 100 experiments were performed with the second method. Figure 4 illustrates the course of fatigue after the muscles have been immersed in solutions of different $CaCl_2$ concentrations. The curves I and II show the fatigue curve of the two muscles when stimulated in the moist chamber. One recognizes that they behave identically. After this they were immersed

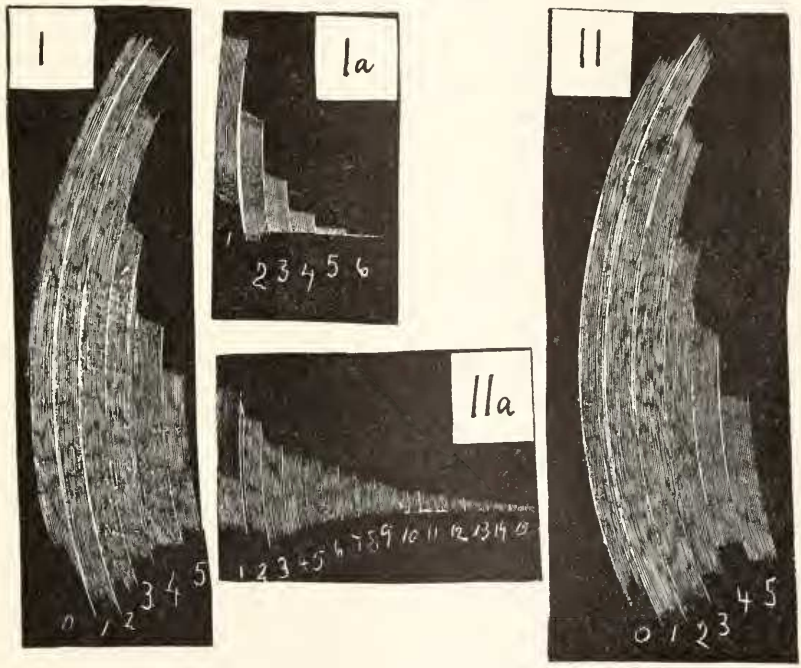

Fig. 4. Stimulation of a pair of sartorii in a moist chamber with maximal condenser discharges 90 times per minute. Then muscle I is immersed in 0.111 M NaCl + 10.2 M $\times 10^{-4}$ KCl + 8.1 M $\times 10^{-4}$ $CaCl_2$ (control fluid) and muscle II in 0.111 M NaCl + 10.2 M $\times 10^{-4}$ KCl + 81 M $\times 10^{-4}$ $CaCl_2$ for nine minutes. Thereafter stimulation in a moist chamber as before (curve I*a*, II*a* respectively). The numbers at the curves indicate the time in minutes after the beginning of stimulation.

for nine minutes in solutions of NaCl + KCL + $CaCl_2$ which were alike save for the $CaCl_2$ concentration. This was 8.1 M $\times 10^{-4}$ in the first case and 81 M $\times 10^{-4}$ in the latter one. Finally they were trans-

ferred again into the moist chamber and stimulated in the same way as in the first series. The experiments yielded the curves I*a* and II*a* and prove that the solution rich in $CaCl_2$ preserved the contractility of the muscle much better than that with the lower $CaCl_2$ concentration. The height of contraction which was observed in the first muscle (treated with 8.1 M $\times$ 10^{-1} $CaCl_2$) after five minutes was the same as

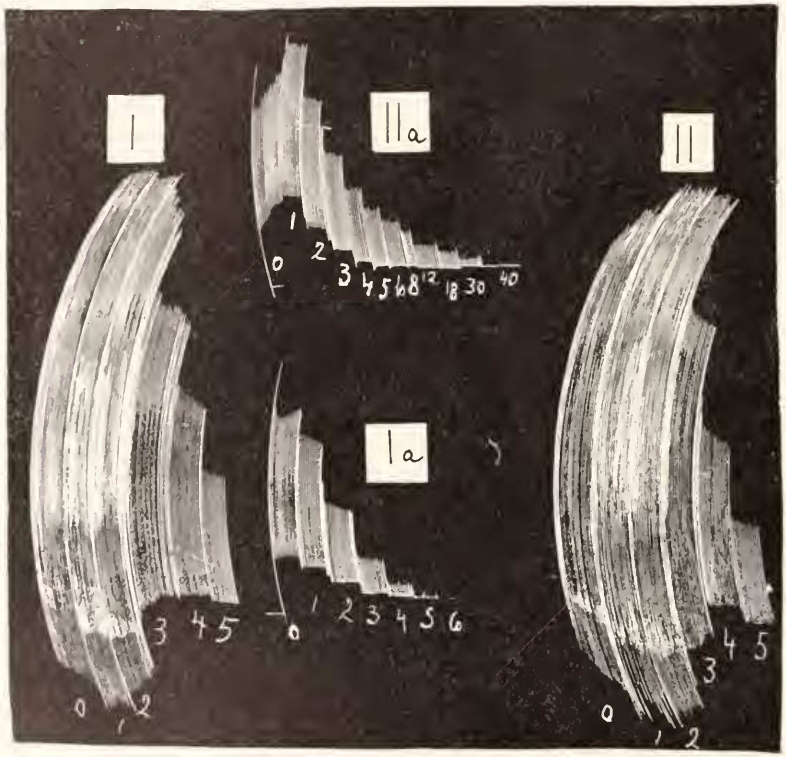

FIG. 5. Procedure as in Fig. 4. Muscle I immersed in control fluid; muscle II in 0.111 M NaCl + 10.2 M $\times$ 10^{-4} KCl + 40.5 M $\times$ 10^{-4} $CaCl_2$.

in the second muscle (treated with 81 M $\times$ 10^{-1} $CaCl_2$) after fourteen minutes.

Another example is reproduced in Fig. 5. It is of particular interest because in the first series of contractions the second muscle shows spontaneously a greater fatigability than the first one. Nevertheless, the treatment of the muscle with the solution containing a higher $CaCl_2$ concentration overcompensated the deficiency, since this muscle remains contractile for 40 minutes and the control muscle for only five

minutes. In a group of experiments the solution with the higher $CaCl_2$ concentration contained the same NaCl concentration as the other one so that the osmotic pressure of the first solution was slightly higher, while in a second group the NaCl concentration was a little lower in order to keep constant the osmotic pressure in spite of the differences in $CaCl_2$ concentration. In both groups the solution rich in $CaCl_2$

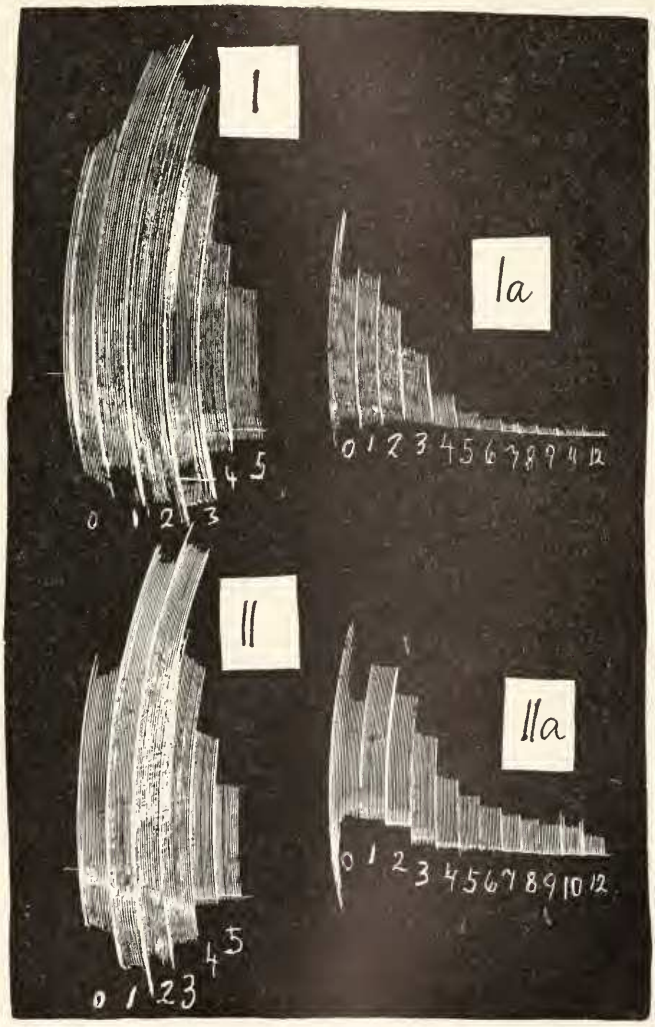

FIG. 6. Procedure as in Fig. 4. Muscle I (Fig. 1 and 1*a*) immersed in control fluid; muscle II in 0.111 M NaCl + 10.2 M $\times 10^{-4}$ KCl + 40.5 M $\times 10^{-4}$ $CaCl_2$ (Fig. II and II*a*).

delayed the fatigue, and this result was also obtained if by addition of $NaHCO_3$ the salt solution were completed to a typical Ringer's solution. Figure 6 shows this in a very striking way because, in spite of the weaker contractions of the second muscle, the fatigue is delayed after the muscle has been immersed in a Ringer's solution with five times its normal $CaCl_2$ concentration.

FIG. 7. Procedure as in Fig. 4. Muscle I in 0.111 M NaCl + 10.2 M × 10^{-4} KCl + 81 M × 10^{-4} $CaCl_2$ (I and I*a*); muscle II in control fluid (II and II*a*).

The favorable effect of the solution with higher $CaCl_2$ concentration in delaying fatigue may be illustrated by Table I, in which the time of the preservation of contractility was determined for each pair of

muscles after they had been immersed in salt solutions with different $CaCl_2$ concentrations.

TABLE I

The Dependence of the Duration of Contractility upon the $CaCl_2$ Concentration

After a previous period of stimulation for five minutes, the muscles were immersed in 0.111M NaCl + 0.00102M KCl + different $CaCl_2$ concentrations as indicated in this table. After this, stimulation was continued in a moist chamber.

No.	$CaCl_2$ Concentration	Duration	$CaCl_2$ Concentration	Duration
		minutes		minutes
1	$8.1M \times 10^{-4}$	5	$40.5M \times 10^{-4}$	> 17
2	"	5	$40.5M \times 10^{-4}$	> 40
3	"	6	$81M \times 10^{-4}$	> 15
4	"	4	$81M \times 10^{-4}$	> 9
5	"	4	$81M \times 10^{-4}$	46
6	"	6	$81M \times 10^{-4}$	20

The experiments with the second method reveal still another fact which seems to be of interest for the problem of the tonus of skeletal muscle. The curves of Fig. 5 in particular show that after the inter-

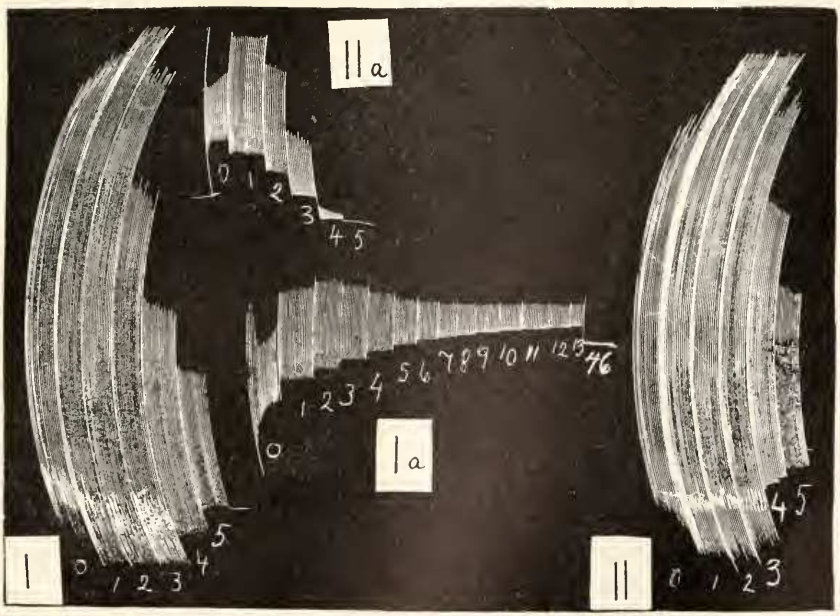

FIG. 8. Procedure as in Fig. 4. Solutions as in Fig. 7.

val during which the muscles have been immersed in salt solutions the stimulation leads to a contracture which is slightly stronger in the Ca-muscle. Furthermore, the diminution of this contracture is delayed in the Ca-muscle.[4] In this experiment the contracture still increased one minute after the beginning of stimulation while in the control muscle it diminished. This behavior is even more pronounced in the experiment illustrated by Fig. 8, in which the Ca-muscle showed an increase in tonus during almost the entire period of stimulation, although the control muscle showed a rapid loss of tonus during this time. The tonus effect of calcium becomes apparent when the $CaCl_2$ concentration is 81 M $\times$ 10^{-4} or higher. In reference to the delay of fatigue the muscles show an unequal behavior. In most cases the fatigue was delayed in spite of the increase in tonus, but in some cases (compare Fig. 7) the tonus increased so rapidly that it led to an acceleration of fatigue.

The behavior of the stimulated and unstimulated muscle in solutions containing different $CaCl_2$ concentrations was compared and it was concluded that stimulation entirely changed the reactivity of the muscle to this particular ion. This fact was explained by the increase in permeability of the muscle. The observations on the effects of calcium ions on the tonus of skeletal muscle seem to support this view since Neuschlosz (1922) observed that the immersion of the *resting* muscle in Ringer's solution with increased $CaCl_2$ concentration lead always to a *decrease* in tonus. It is probable that the muscle brought into a state of high permeability by previous stimulation takes up $CaCl_2$ in a greater amount than the resting muscle and this leads to entirely different phenomena. It may be emphasized that about the same concentrations were used in Neuschlosz' experiments and our own. If the interpretation is correct it is to be expected that far greater concentrations of $CaCl_2$ would be able to increase the tonus in the resting muscle. In fact it was found by Guenther (1905) that $CaCl_2$ in one per cent solution leads to an increase in tonus which occurs after a latent period of several hours. Probably the irritability of the muscle had faded under these conditions. In contrast to that, the experiments described above show that $CaCl_2$ may immediately raise the tonus of the irritable muscle provided that the permeability had been increased by previous stimulation. Therefore not only by K as Neuschlosz found, but also by Ca the tonus of muscle can be increased without decrease of irritability.

Leaving aside the effects of $CaCl_2$ on the tonus of muscle, the ex-

[4] For the sake of brevity the expression Ca-muscle is used for the muscle which has been immersed in the solution containing the higher $CaCl_2$ concentration.

periments lead to the conclusion that increase in $CaCl_2$ concentration through a very wide range reduces the higher degree of permeability produced by stimulation, and therefore delays fatigue. Further experiments dealt with the question whether the Ca effect is specific or whether it is replaceable by other cations. The effects of Sr, Mg and Ba were examined.

The experiments showed that *none of the alkali earths can replace calcium.* Rather small concentrations of Sr, Ba and Mg had only a slight effect if any, and greater concentrations accelerated fatigue. $MgCl_2$ seemed to have the most harmful effect, since it reduced the life duration of the muscle which had been previously immersed in a solution of $NaCl + KCl + MgCl_2 + NaHCO_3$, while the effect of Sr and Ba was mostly limited to a decrease in the height of concentration. The concentrations used are given in Table II.

TABLE II

Each solution ordinarily contains beside the salts with bivalent cations 0.111M $NaCl + 10.2M \times 10^{-4}$ $KCl + 12.5M \times 10^{-4}$ $NaHCO_3$.

	Concentration	Effect on Fatigue
Mg	$15.4M \times 10^{-4}$	—
	$23.1M \times 10^{-4}$	—
	$30.8M \times 10^{-4}$	—
Ba	$23.9M \times 10^{-4}$	—
	$31.8M \times 10^{-4}$	—
Sr	$16.7M \times 10^{-4}$	—
	$32.5M \times 10^{-4}$	—
	$40.7M \times 10^{-4}$	—
	$81.4M \times 10^{-4}$	—

In another series one muscle was immersed in a solution of $NaCl + KCl + CaCl_2 + NaHCO_3$, the composition of which was identical with that used for the control muscle save for the addition of $SrCl_2$, $BaCl_2$ or $MgCl_2$. In these experiments it was found that all three cations were able to delay the fatigue of the muscle but the effect was much less than in the experiments described above in which an excess of $CaCl_2$ was studied. Furthermore, it was found that the favorable effect was limited to a very small range of concentrations. When this was surpassed the fatigue was accelerated. The quantitative data are reproduced in Table III.

TABLE III

The Effect of Sr, Ba and Mg on the course of fatigue if added to 0.111M NaCl + 10.2M × 10^{-4} KCl + 8.1M × 10^{-4} $CaCl_2$ + 12.5M × 10^{-4} $NaHCO_3$.

	Concentration	Effect on Fatigue *
Sr	32.5M × 10^{-4}	+
	40.7M × 10^{-4}	+
Ba	19.8M × 10^{-4}	+
	23.9M × 10^{-4}	+
	31.8M × 10^{-4}	−
Mg	15.4M × 10^{-4}	+
	19.2M × 10^{-4}	±
	30.8M × 10^{-4}	−

* + indicates delay in fatigue; −, acceleration in fatigue; ±, no change.

DISCUSSION

The experiments described in this paper prove that the laws of ion-antagonism differ quantitatively in the resting and the stimulated muscle. The increase in permeability in the latter leads rapidly to fatigue and this can be delayed when an excess of Ca is added which reduces permeability. On the other hand, a Ringer's solution containing an excess of $CaCl_2$ is unfavorable for the preservation of irritability in *resting* muscle. The fundamental difference in the behavior of resting and stimulated muscle in reference to ions is further illustrated by experiments in which the effect of other bivalent cations was studied. It was found that Sr, Mg or Ba added to Ringer's solution with normal $CaCl_2$ concentration exerted a favorable influence on the stimulated muscle, that is, the fatigue was delayed. But the same solutions were unable to preserve the irritability of the unstimulated muscle as well as Ringer's solution. The experiments permit one to conclude that *the composition of the optimal salt solution is not a definite one for a certain type of cell but is dependent upon its degree of permeability.*

This implies the fact that the efficiency of ions differs quantitatively if applied to a cell in a state of low or high permeability. The experiments on the tonus effect of calcium described above show the correctness of this conclusion. The increase in tonus observed in the stimulated muscle begins immediately after stimulation, while, according to Guenther, it requires a Ca concentration ten times higher and several hours in resting muscle.

The experiments throw an interesting light upon the specificity of ion antagonism.[6] They indicate that, in contrast to the ion antagon-

[6] Compare Gellhorn, 1924 and 1926.

ism between K and bivalent cations in muscle in which numerous cations are of great antagonistic efficiency, calcium cannot be replaced by any other bivalent cation in its effect of delaying fatigue. This is astonishing because the suppression of the K contracture by bivalent cations is based upon their power to reduce permeability (Gellhorn, 1928). The writer found recently in experiments on vital staining in sea urchin eggs that the permeability of the cell to stains could be prevented by Ca but not by Mg, Sr or Ba (1931). But the explanation of the specificity seems to be quite different in sea urchin eggs and muscle, since in the former Sr, Ba and Mg were unable to decrease permeability, while in muscle permeability is decreased. This is not only indicated by observations on K-contracture but also by the fatigue experiments of this paper, since it was shown that addition of Sr, Ba or Mg to Ringer's solution containing $CaCl_2$ was effective in reducing fatigue. The reason why Ca cannot be entirely replaced by Sr, Ba and Mg seems to lie in the fact that Ca is probably the only ion which diminishes the increase in permeability of muscle in a completely reversible manner. However, when coarser changes in the surface of the cell are concerned, such as are produced by K, several bivalent cations act antagonistically. Fewer of these ions are effective if irritability is concerned, as in paralysis of muscle (Höber, 1917), than in the case of K-contracture, which is rather independent of irritability. In the former case Ca is the most antagonistic ion, in the latter case the heavy metals (Gellhorn), because here the greater the power in reducing permeability, the more efficiently is the contracture suppressed. The impossibility of fine reversible changes in permeability, which are necessary for the preservation of irritability, is not concerned here. The intermediate case, the restoration of irritability after K-paralysis, is influenced in a somewhat intermediate manner: calcium is most effective, but other bivalent cations are effective to a lesser degree. The importance of calcium for the preservation of irritability and contractility is apparent. It is conceivable that the delay of fatigue requires a still finer adjustment than the partial restoration of irritability after K-paralysis. This may be the reason for the specificity of the calcium effect in the fatigue experiments of this paper.

Summary

1. The fatigue of the sartorius muscle of the frog can be delayed by an excess of calcium chloride in Ringer's solution, although this solution does not preserve irritability of resting muscle as well as Ringer's solution. Therefore, in contrast to the view generally accepted today, *the quantitative composition of the optimal solution depends upon the permeability of the cell.*

2. This conclusion implies a quantitative difference in the efficiency of ions in relation to permeability. This is illustrated by observations on the increase of muscle tonus in solutions with high $CaCl_2$ content. The change in tonus takes place in irritable muscle.

3. The effect of calcium on fatigue is specific. Neither Sr, Ba nor Mg can replace it. But these cations can delay the fatigue, although to a lesser extent than calcium, when added to Ringer's solution containing 8.1 M $\times$ 10^{-4} $CaCl_2$. The cause of this specificity is discussed.

BIBLIOGRAPHY

Embden, G., and Adler, E., 1922. *Zeitschrift f. physiol. Chemie*, **118**: 1.

Gellhorn, E., 1924. In Oppenheimer's *Handbuch der Biochemie*, **2**: 98.

Gellhorn, E., 1926. Neuere Ergebnisse der Physiologie. Leipzig, Chapter 2.

Gellhorn, E., 1928. *Pflüger's Arch.*, **219**: 761.

Gellhorn, E., 1929. Das Permeabilitätsproblem, seine physiologische und pathologische Bedeutung. Berlin.

Gellhorn, E., 1930. Contributions to Marine Biology, p. 115. Stanford University Press.

Gellhorn, E., 1931. *Protoplasma*, **12**: 66.

Guenther, A. E., 1905. *Am. Jour. Physiol.*, **14**: 73.

Höber, R., 1917. *Pflüger's Arch.*, **166**: 531.

Neuschlosz, S. M., 1922. *Pflüger's Arch.*, **196**: 503.

Scheminzky, F., 1928. *Zeitschr. f. Biol.*, **87**: 189.

Scheminzky, F., 1930. *Pflüger's Arch.*, **225**: 303.

Weiss, H., 1922. *Pflüger's Arch.*, **196**: 393.